

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Rehab Al-Jamal and David Harrison
Title: "Tissue Repair by Modulation of Beta-1 Integrin Biological Function"
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DECLARATION UNDER 37 C.F.R. § 1.132 OF REHAB AL-JAMAL

I, REHAB AL-JAMAL, do hereby declare:

1. I obtained the degree of BSc (Hons) in Biomedical Sciences/Pharmacology and Toxicology (1993, Bradford University-UK) and PhD in the interaction between lung tissue mechanics, extracellular matrix proteoglycans and mechanical forces where the main focus was on lung tissue remodelling during injury (2001, Experimental Medicine/Meakins Christie Laboratories, McGill University-Canada). I have received training in pharmacology at SmithKline Beechams (1992). I have held a post-doctoral position (2001-2003) at Edinburgh University focusing on the role of integrins in cartilage mechanotransduction and how remodelling in osteoarthritis affects integrin function. Since 2003, I have held the position of co-principal investigator then principal investigator working on mechanisms of tissue injury, remodeling and repair. I have extensive experience in the field of tissue remodelling and repair as evident

from an award for distinguished PhD student (McGill University, 2000) and continual project funding awarded by the Scottish Government (2 major project award 2003-2007 and 2008-2011) in addition to Edinburgh University departmental awards (2002-2003 and 2007-2008). I am the author and co-author of 5 major scientific publications which relate to the field of tissue remodelling and repair. I have given invited talks on the subject at Novartis (Horsham, UK), the Swiss Federal Institute (Lausanne, Switzerland) and BIT's 2nd International Congress of Antibodies-2010 (Beijing, China). In 2008, I was invited by the journal Pharmacology and Therapeutics to write a review detailing the role of beta1 integrin in tissue repair (Pharmacol Ther. 120(2):81-101.PMID: 18708090).

2. I am one of the inventors on US Patent Application Serial No. 10/576,274.

FIRST EXPERIMENT - Measurement of MMP12 in Bronchoalveolar Lavage Fluid (BAL)

3. Under my supervision, the JB1a antibody, which is known to modulate function of beta 1 integrin by binding to the beta 1 integrin molecule in a region of amino acid residues 82 to 87 comprising residues TAEKLLK (SEQ ID NO:1) of the sequence of the mature beta 1 integrin molecule resulting in (i) an inhibition of the apoptotic pathway, (ii) an alteration in the metalloproteinase balance and (iii) an increase in the anabolism of the extracellular matrix, was administered to mice after the induction of stable emphysema using porcine pancreatic elastase using the following procedure:

3.1 Female C57/BL6 mice (6-8 weeks old) were instilled intra-tracheally with porcine pancreatic elastase (PPE) as detailed on page 55, line 28 to page 56, line 1 of the application as filed. All procedures were approved by the Home Office and the Institutional Ethics Committee. At day 14 or 21, mice were treated intra-tracheally with the anti-integrin antibody JB1a at 3mg/kg in sterile PBS. The dose chosen is equivalent to the dose of clinically used antibodies against alpha4 beta1 integrin (Miller DH, et al. 2003). A control group was instilled initially with PBS and at day 14 or 21 with PBS. For the group treated at day 14, the animals were terminated at day 21 (21day group) and the group treated on days 21 and 28 were terminated at day 35 (35 day group) as follows.

The animals were anaesthetised using sodium pentobarbitone (45mg/kg), paralysed using pancuronium bromide (0.8mg/kg) and tracheostomised and ventilated using a small animal ventilator (Flexivent, SCIREQ, Montreal) at 8ml/kg and a rate of 150 breaths/minute and positive end expiratory pressures (PEEP) of 3.5 cmH₂O.

The pressure-volume curve was obtained during inflation and deflation in a stepwise manner by applying volume perturbation incrementally during 16 seconds. The pressure signal was recorded and the pressure-volume (P-V) curve calculated from the plateau of each step. The constant K was obtained using the Salazar-Knowles equation and reflects the curvature of the upper portion of the deflation P-V curve. Quasi-static elastance reflects the static elastic recoil pressure of the lungs at a given lung volume. It was obtained by calculating the slope of the linear part of P-V curve.

After the measurements, the animals were sacrificed and bronchoalveolar lavage collected by 3 rinses using sterile LPS-free saline. The bronchoalveolar lavage was centrifuged at 2000 rpm for 10 minutes and the supernatants lyophilised and separated on 10% SDS-PAGE, transferred onto Hybond-ECL (GE Healthcare) and probed for metalloproteinase-12 (clone M19, polyclonal goat IgG, Santa Cruz) followed by HRP labelled secondary antibody and developed using ECL-Plus (GE Healthcare) and exposed to Hyperfilm ECL (GE Healthcare).

RESULTS

4. Results are shown in Figure 1 of Appendix A. PPE increased the levels of MMP12 only in the 35 day group. Modulation of beta1 integrin by targeting the TAEKLLK sequence using JB1a modulated the effects of PPE on MMP12 by almost abolishing MMP12 only in the 21 day group. Taken together within the context of time, modulation of beta1 integrin modulated the activity of MMP12 which occurred during the process of injury.

CONCLUSION OF FIRST EXPERIMENT

5. Based on the data presented in Figure 1 of Appendix A, I conclude that targeting the TAEKLLK amino acid residues of beta 1 integrin had an effect on MMP12 expression in emphysematous mice.

SECOND EXPERIMENT - Measurements of MMP2/9 (Gelatinases) and MMP12 (Elastase) in Human Mesenchymal and Epithelial Cell Co-culture in vitro

6. Under my supervision, the JB1a antibody, which is known to modulate function of beta 1 integrin by binding to the beta 1 integrin molecule in a region of amino acid residues 82 to 87 comprising residues TAEKLLK (SEQ ID NO:1) of the sequence of the mature beta 1 integrin molecule resulting in (i) an inhibition of the apoptotic pathway, (ii) an alteration in the metalloproteinase balance and (iii) an increase in the anabolism of the extracellular matrix, was added to adult human lung fibroblasts using the following procedure:

6.1 Adult human lung fibroblasts (CCD-8Lu) were seeded onto collagen I coated BioFlex 6 well plates at 0.5×10^6 /well. The following day, NCI-H441 were seeded on top of the fibroblasts at the same density. Cells were starved with media containing 0.1% FCS. The plates were subjected to stretching at 2-10% sinusoidal stretch at 1Hz for 2, 4 or 6 hours. PPE was added at 0.3U/ml alone or in combination with JB1a (1µg/ml). At the end of the stretch, the media was aspirated, snap frozen and lyophilised and enzyme activities assayed using EnzChek® Gelatinase/Collagenase and EnzChek® elastase assay kits (Invitrogen) according to the manufacturers' instructions.

RESULTS

7. Results are shown in Figures 2 and 3 of Appendix A as follows:

PPE increased the activity of elastase from 2 hours after exposure which peaked at 4 hours. Targeting beta1 integrin's TAEKLLK sequence reduced elastase activity by ~50% only at the 4 hour time point (Figure 2).

PPE increased gelatinase activity in a biphasic manner over time (2 and 6 hours). Targeting the TAEKLLK sequence of beta1 integrin reduced the PPE-induced increase in

gelatinase activity at the 2 and 6 hour time points. However, at the 4 hour time point, targeting the TAEKLLK amino acid residues of beta 1 integrin increased PPE-induced gelatinase activity (Figure 3).

CONCLUSION OF SECOND EXPERIMENT

8. Based on the data presented in Figures 2 and 3 of Appendix A, I conclude that targeting the TAEKLLK amino acid residues of beta 1 integrin produced a biphasic effect on MMP 2, 9 and 12 in emphysematous adult human lung fibroblasts.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 9th day of Sep 2010.



REHAB AL-JAMAL, Ph. D.

Declaration under 37 CFR § 1.132 of Rehab Al-Jamal

Appendix A

Figure 1. The Effect of PPE and JB1a on MMP12 in BAL fluid

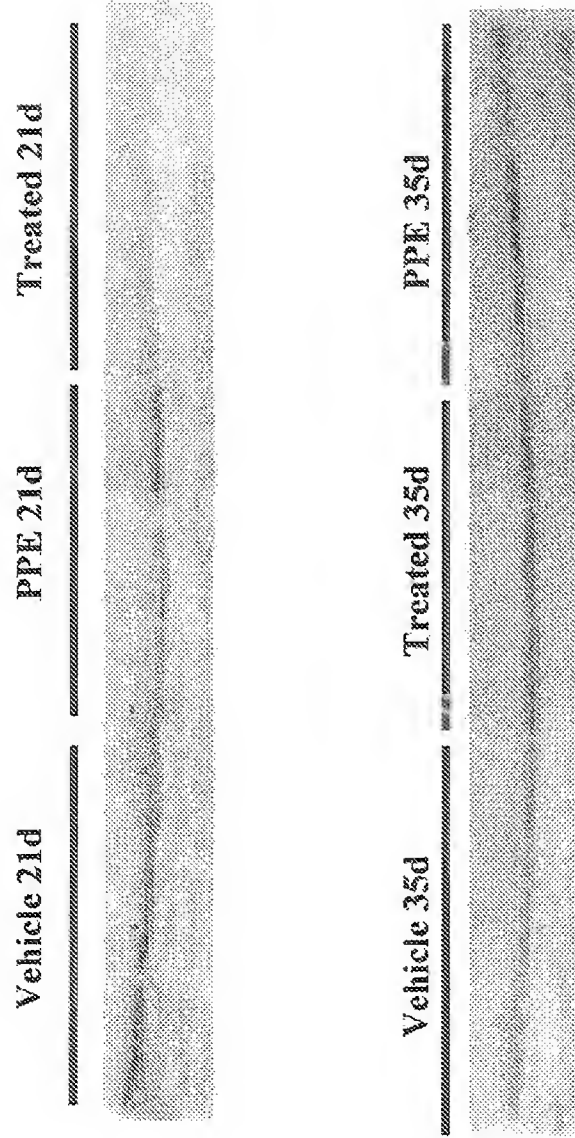


Figure 2. The effect of elastase induced injury in vitro on elastase activity

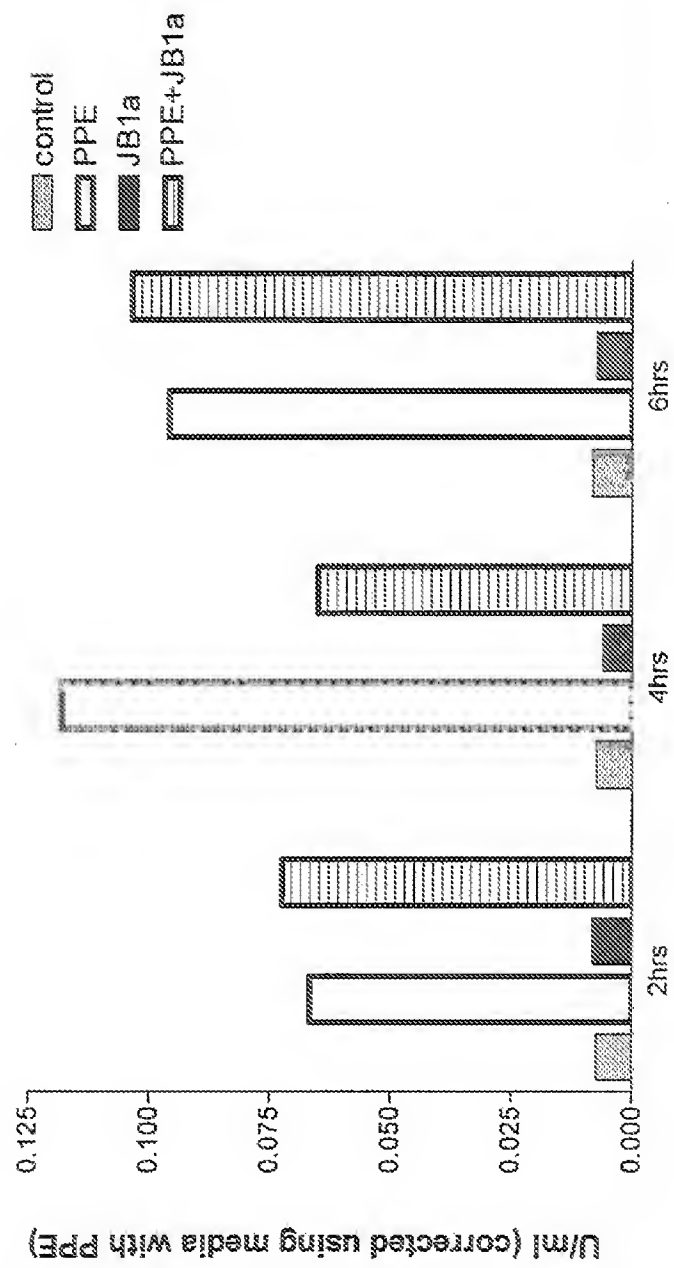


Figure 3. The effect of Gelatinase (MMP2 and 9) induced injury in vitro on elastase activity

